Occurrence of infection with *Toxoplasma gondii* and factors associated with transmission in broiler chickens and laying hens in different raising systems[1]

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ABSTRACT.- Millar P.R., Alves F.M.X., Teixeira V.Q., Vicente R.T., Menezes E.M., Sobreiro L.G., Pereira V.L.A. & Amendoeira M.R.R. 2012. **Occurrence of infection with *Toxoplasma gondii* and factors associated with transmission in broiler chickens and laying hens in different raising systems.** *Pesquisa Veterinária Brasileira 32(3):231-236.* Laboratório de Toxoplasmose, Instituto Oswaldo Cruz, Fiocruz, Avenida Brasil 4365, Manguinhos, Rio de Janeiro, RJ 21040-900, Brazil. E-mail: amendoei@ioc.fiocruz.br

Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii.* The aim of the present study was to determine the occurrence and identify the risk factors associated with transmission of *T. gondii* to chickens raised in different systems (free-ranged and confined) to produce eggs or meat. The 810 animals were allocated in two experimental groups according to the production system purpose: 460 broiler chickens (Group 1) and 350 layer chickens (Group 2). In order to analyze the possible factors involved in *T. gondii* infection in the chickens, an epidemiological questionnaire was developed for all properties.The serological detection of anti-*Toxoplasma gondii* antibodies was performed by Indirect Immunofluorescence (IFAT) and by Enzime Linked Imunossorbent Assay (ELISA). Since the agreement index (kappa) between these two serological techniques was considered high, 21.2% of the 810 animals were considered reactive. In Group 1, 12.2% (56/460) were positive, while in the Group 2 the positivity rate was 33.1% (116/350). The production system may be influencing the seropositivity of the animals in both groups. However, only in Group 2 it was possible to notice a statistically significant relationship between the breeding system and the frequency of positive sera. This result indicates that, at least for laying hens, the production system is directly involved in *T. gondii* infection. The contact with cats in Group 1 did not influence the distribution of seroreactive animals, but in Group 2 a significant relationship was observed. The occurrence of anti-*T. gondii* antibodies was high in both groups (broiler and posture chickens). Free-ranged chickens raised for egg production proved to be the most exposed group to the *T. gondii* infection. This can be related to the fact that these animals stay for longer periods in the farms, in direct contact with possibly contaminated soil by the presence of domestic cats.

INDEX TERMS: *Toxoplasma gondii,* broiler chickens, laying hens, transmission.

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RESUMO.- [**Ocorrência da infecção por *Toxoplasma gondii* e fatores associados à sua transmissão em aves de corte e postura produzidas em diferentes tipos de criação.**] A toxoplasmose é uma zoonose causada pelo protozoário *Toxoplasma gondii*. O objetivo deste estudo foi determinar a ocorrência e identificar os fatores de risco associados à transmissão de *T. gondii* para frangos criados em diferentes sistemas (caipira e confinado) para produzir ovos ou carne. Os 810 animais foram divididos em dois grupos experimentais de acordo com o propósito do sistema de produção: 460 frangos de corte (Grupo 1) e 350 galinhas poedeiras (Grupo 2). A fim de analisar os possí-

veis fatores envolvidos na infecção pelo *T. gondii* nas galinhas, um questionário epidemiológico foi respondido por todos os proprietários. A detecção sorológica de anticorpos anti-*Toxoplasma gondii* foi realizada pela técnica de imunofluorescência indireta (RIFI) e Enzime Linked Assay Imunossorbent (ELISA). Uma vez que o índice de concordância (kappa) entre estas duas técnicas sorológicas foi considerada alta, 21,2% dos 810 animais foram considerados reativos. No Grupo 1, 12,2% (56/460) foram positivos, enquanto no Grupo 2 a taxa de positividade foi de 33,1% (116/350). O sistema de produção pode estar influenciando a soropositividade dos animais em ambos os grupos. No entanto, apenas no Grupo 2, foi possível notar uma relação estatisticamente significativa entre o sistema de produção e da freqüência de soros positivos. Este resultado indica que, pelo menos para as galinhas poedeiras, o sistema de produção está diretamente envolvido na infecção pelo *T. gondii*. O contato com os gatos no Grupo 1 não influenciou a distribuição dos animais sororreagentes, mas no Grupo 2 uma relação estatisticamente significativa foi observada. A ocorrência de anticorpos anti-*T. gondii* foi alta nos dois grupos (frangos de corte e postura). Galinhas cairpiras criadas para produção de ovos provou ser o grupo mais exposto à infecção *T. gondii*. Isto pode estar relacionado ao fato de que estes animais ficam por períodos mais longos nas fazendas, em contato direto com o solo possivelmente contaminado pela presença de gatos domésticos.

TERMOS DE INDEXAÇÃO: *Toxoplasma gondii*, frango de corte, galinhas poedeiras, transmissão.

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii*. It is a cosmopolitan disease that infects humans and other warm-blooded species. Felids, the definitive hosts, are the only animals that disseminate oocysts to the environment. Other species, including humans, can be infected only by asexual stages, acting as intermediate hosts. In this case, the transmission of the protozoosis occurs vertically by transplacental transmission or when the intermediate host is ingested by other potential host. (Amendoeira et al. 1999, Millar et al. 2008). The role of domestic chickens (*Gallus gallus domesticus*) in the transmission of *T. gondii* were investigated only in a few studies. Due to the rare presence of the parasite, the domestic chicken was not considered an important source of infection for humans (Dubey et al., 2010); however, others studies demonstrated that the lack of personal hygiene procedures during in natura meat manipulation and raw eggs consumption were risk factors for human infection by *T. gondii* (Literak & Hejlicek 1993, Amendoeira 1995, Garcia et al. 2000, Bonna et al. 2006, Millar et al. 2008). Birds, as well as rodents, are considered important intermediate hosts of *T. gondii* because they can be a source of infection for cats. Besides that, as chickens can feed from the ground, they are a good indicator of prevalence of *T. gondii* in the environment, therefore, they are used as sentinel animals in areas where the prevalence rate of human infection is high (Dubey et al. 2002, Dubey 2010). The clinical symptoms of toxoplasmosis in birds, even those infected with virulent strains, are rarely reported (Hepding 1939, Fankhauser 1951, Nobrega et al. 1967, Dubey et al. 2002, Dubey et al. 2006), thus chickens are considered resistant to clinical toxoplasmosis. Torticollis, inability to stand, lateral recumbency and anorexia are clinical signs already described (Dubey et al. 2007, Dubey 2010).

Chickens raised confined have a low rate of infection because management, containment, and proper hygiene procedures reduce or even avoid the contact of animals with the infection sources of the parasite. However, the large consumer demand for products with high quality standards influenced changes in the production systems used to raise chickens. Nowadays, consumers are also more concerned about animal welfare and therefore demand production systems that reduce animal suffering. Thus, free-ranged and organic systems are increasing rapidly worldwide. Although animal health is a critical part of animal welfare, evidences suggest that the health of the animals raised in free-range and organic farms is not always better than that observed in animals raised in conventional livestock production systems. The aim of the present study was to determine the occurrence and identify the risk factors associated with transmission of *T. gondii* to chickens raised in different systems (free-ranged and confined) to produce eggs or meat.

MATERIALS AND METHODS

In the present study, 810 domestic chickens (*Gallus gallus domesticus*) from Rio de Janeiro state were used. The animals were allocated in two experimental groups according to the production system purpose: 460 broiler chickens (Group 1) from 10 farms, slaughtered under sanitary inspection and ranging from 42 to 90 days of age, and 350 layer chickens (Group 2), from nine properties, ranging from 6 and 18 months of age.

In both groups there were animals raised in confinament system, free-ranged system and organic system.

All farms had veterinary care and were registrated in agencies responsible for monitoring. The farms with organic production had a quality certification from the Biological Producers Association of the state of Rio de Janeiro (ABIO).

Blood samples from broiler chickens were collected by veterinarians at the moment of animal bleeding. Blood samples from layer chickens were collected by veterinarians in the properties. Sera were stored at -20°C until serological analysis in the Laboratory of Toxoplasmosis of the Oswaldo Cruz Institute, Rio de Janeiro, Brazil.

The sample population, calculated by Epi-Info version 6.0., was random and stratified by type and purpose of production system according to Thrusfield (1986), for an estimated prevalence of 10% for Group 1 and 30% for Group 2, with an absolute precision of 5% level and of 95%.

The serological detection of anti-*Toxoplasma gondii* antibodies was performed by Indirect Immunofluorescence (IFAT) (Camargo 1964) and by Enzime Linked Imunossorbent Assay (ELISA) (Voller et al. 1976). For IFAT and ELISA, the *conjugated fluorescein* isothiocyanate (FITC -, Sigma-Chemical®, Brazil) and *Peroxidase Conjugated* Anti-*Chicken IgG (*Sigma-Chemical®, Brazil*) were used, respectively. T*achyzoites of the RH strain of *T. gondii*, maintained in house, were used for the antigen production.

Table 1. Distribution of broilers chickens and laying hens according to the results of the ELISA and raising system

	Broiler chickens (Group 1)			Laying hens (Group 2)		
	Reagents	Not reagents	Total	Reagents	Not reagents	Total
Raising system	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Confinement chickens	22 (9,6)	208 (90,4)	230 (100)	26 (14,8)	149 (85,2)	175 (100)
Free-ranged chickens	34 (14,8)	196 (85,2)	230 (100)	90 (51,4)	85 (48,6)	175 (100)
Total	56 (12,2)	404 (87,8)	460 (100)	116 (33,1)	234 (66,9)	350 (100)

Broiler Chickens: $\chi^2 = 2.92$ (p=0.0871).
Laying hens $\chi^2 = 52.815$ (p<0.0001); OR= 0,16 (0.09 a 0.27).

In IFAT, sera were screened at 1:16, 1:64, 1:256, 1:1024 and 1:4096 dilutions, and titers higher or equal to 16 were considered positive. In ELISA, the serum was diluted to 1:100 and the readings at or above the cut-off value of the plate were considered positive.

The analysis of risk factors were performed by the application of questionnaires consisting of objective questions: (*i*) type of production system (confined or free-ranged chickens), (*ii*) presence of cats and rodents in the farm, (*iii*) type of rodent control and water supply of the farm and (*iv*) whether the owners and employees had the habit of consuming meat or eggs from their own farm.

The real assessment of agreement between the serological techniques was calculated by the inter-rater *agreement* statistic *Kappa* with 95% confidence interval. For the analysis of the variables and the frequency of infection it was used the chi-square (c 2), 5% significance level. The odds ratio was used to measure the strength of association between the statistically significant variables and the level of *T. gondii* antibodies found. Both the chi--square and the odds ratio were performed with the Biostat 2.0 software.

This study was approved by Animal Research Ethical Committee of FIOCRUZ (P0325/07).

RESULTS

The percentage of animals positive for *Toxoplasma gondii* by IFAT and ELISA was 17.2% (139/810) and 21.2% (172/810) respectively. The agreement index (kappa) between these two serological techniques was considered high (k = 91.4). Therefore, sera were considered reactive when ELISA was positive (21.2%), since there were no results only IFAT reagents. In Group 1, 12.2% (56/460) of the animals were positive for *T. gondii*, and 33.1% (116/350) of the animals of Group 2 were positive.

Based on the epidemiological questionnaire results, it was possible to determine if the livestock production system (confinement and free-ranged chickens) influenced the seropositivity of the animals in both groups.

In Table 1, it was possible to notice that Group 1 had a higher percentage of positive individuals raised in free--range system; however, the relationship between this variable and the frequency of reactive sera was not statistically significant. In Group 2, free-ranged chickens showed a higher percentage of positive animals (51.4%, n=90) when compared to birds raised confined (14.8%, n=26). The relationship between the production system and the frequency of positive sera was very strong, reaching a significant value of chi-square. This result indicates that, at least for laying hens, the production system is directly involved in *T. gondii* infection.

The serological IFAT results showed that in Group 1, 7.8% (n=18) of the 230 confined chickens were seroreactive, being 1:16 (n=14, 77.7%) the most common titration, followed by 1:64 (n=4, 22.2%). None of these chickens presented titers equal to or greater than 1:256. For the 230 free-ranged broiler chickens, 11.3% (n=26) were positive for *T. gondii* by IFAT, being the 1:16 the most common titration (n=20; 76.9%), followed by 1:64 (n=5; 19.2%), and 1:256 (n=1, 3.9%) (Fig.1). In Group 2 the results revealed that 18 (10.3%) of the 175 confined chickens were IgG seroreactive, being 1:16 (n=16; 88.9%) the most common titration, followed by 1:64 (n=2; 11.1%), and none of these animals presented titers equal to or greater than 1:256. On the other hand, for the 175 free-ranged laying chickens, the results indicated that the titration was more homogeneous and higher. The seroreactive animals (n=77, 44.0%) had titers ranging from 1:16 to 1:4096. The most frequent was 1:64 (n = 20, 26.0%), followed by 1:256 (n=19, 24.7%), 1:16 (n=15, 19.5%), 1:1024 (n=13, 16.8%), and 1:4096 (n=10, 13.0%) (Fig.2).

Owners were asked about the presence of cats in their farms and consequently about the contact of these animals with the chickens. Table 2 shows that, in Group 1, the contact with cats did not influence the distribution of seroreactive animals. In Group 2, 7 of 9, farmers reported the presence of cats, and a significant relationship between contact with these animals with the chickens and the number of those positive sera were observed.

In all 10 farms from Group 1, the presence of rodents was reported. The control of these animals in eight farms is performed by active methods (chemicals, traps, des-

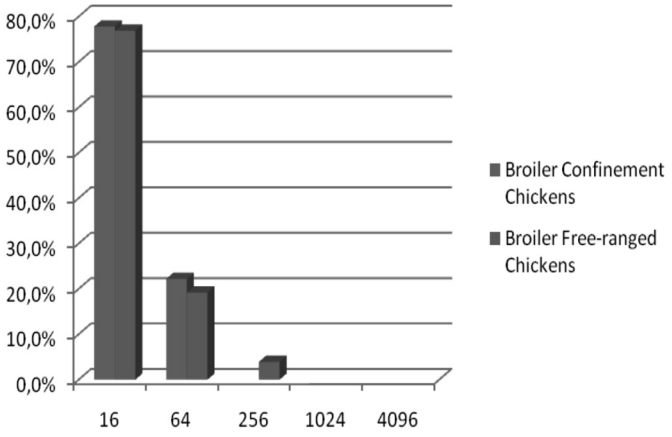

Fig.1. Percentage of specific IgG antibody titers to *Toxoplasma gondii* found in confinement and and free-ranged broilers chickens seropositive by indirect immunofluorescent reaction

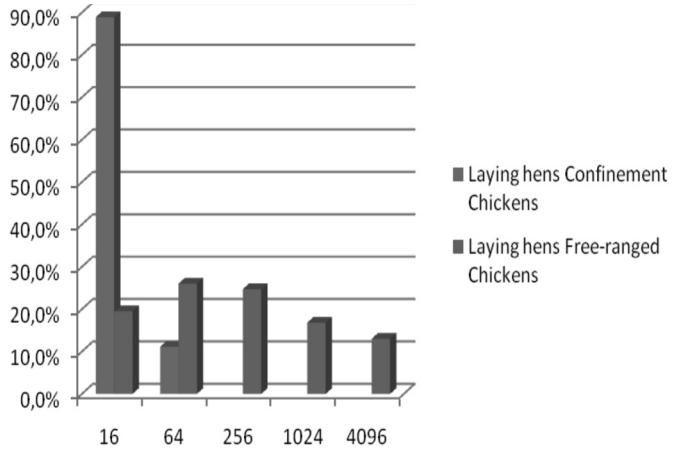

Fig.2. Percentage of specific IgG antibody titers to *Toxoplasma gondii* found in confinement and and free-ranged laying hens seropositive by indirect immunofluorescent reaction

truction of burrows) while in two farms passive methods (cats) are used. In Group 2, only one farm reported absence of rodents, and passive control of these animals is used in most properties. Public water supply was not present in any farm. In both groups, the water came from water wells and/or sources. In Group 1, owners and employees of 8 farms reported the consumption of chicken meat, while in Group 2 all egg-producing farmers reported that they frequently eat both meat and eggs.

DISCUSSION

The occurrence of *Toxoplasma gondii* antibodies observed in this study can be considered high. In broiler chickens this may be due to the free-range system which allowed outdoor access for farm animals, resulting in higher risk of transferring zoonotic pathogens as *T. gondii*. Araújo et al. (1989) in Rio Grande do Sul observed a prevalence of 2.8% in broiler confinement chickens by indirect hemagglutination test, while Garcia et al. (2000) in Paraná had 6.5% positivity in broiler chickens in the IFAT assay and Meireles et al. (2003) in São Paulo could not identify the *T. gondii* in any of the 185 chickens studied by ELISA. However, the comparison of these researches' results must be done with caution because many studies with broiler chickens are made with animals raised in confinement, using different tests and with animals from different regions.

Garcia et al. (2000) discuss the role of broilers raised on an industrial scale, considering them of minor importance in *T. gondii* transmission to humans, not only because of the fast raising system, but also because it did not allow contact with cats. However, the results presented in this paper show that, depending on the management and sanitary conditions of the farm, the broilers can and should be considered as a source of infection. Several studies describe the isolation of the parasite not only in muscles but also in organs such as heart, liver and gizzard (Kaneto et al. 1997, Dubey et al. 2002, Dubey et al. 2006). This fact, associated with inadequate cooking of meat and/or viscera, as in microwave and grills, reinforce this assertion.

As the broiler chickens in Group 1, the laying hens in Group 2 also showed a high percentage of seroreactive individuals, corroborating with literature findings (Garcia et al. 2000, Silva et al. 2003, Bonna et al. 2007, Dubey, 2010). The frequency of positive chickens in this study would possibly be higher if half of the animals were not raised in confinement.

Table 1 shows a lower percentage of positive animals in intensive chicken farming, which was expected in this case, since the confinement, management and certain hygiene measures reduce or even extinguish the contact with sources for *T. gondii* infection. The same was true for free-ranged chickens, since they spend some time inside the sheds, have free access to grazing areas and therefore were more susceptible to the contact with different infection sources of the parasite. Moreover, the animals in this latter group were slaughtered later, aged about three months old, which increased the chance of getting into contact with possible sources of infection.

However, it is worth mentioning the fact that farms in Group 2 were controlled properties, having constant medical and veterinary assistance and also satisfactory management, which probably contributed to maintain a low rate of seropositive birds.

There are no reports in literature about the *T.gondii* infection in egg-producing farms, where birds are typically housed in rows of battery cages. However Garcia et al. (2000) observed in Paraná 11.9% positive free-ranged chickens by IFAT, much lower than the observed in this study (51.4%). One explanation for this could be the different levels of environmental contamination in both places, since the technique used was the same in both studies. In Barra Mansa, rural region of Rio de Janeiro State, Bonna et al. (2007), also by IFAT, identified a prevalence of 47.8% positive chickens, a very close result to the observed in this research. Still in Rio de Janeiro State, in Campos dos Goytacazes, Silva et al. (2003) demonstrated, by the modified agglutination test (MAT) a higher positive percentage

Table 2. Distribution of broilers chickens and laying hens according to the results of the ELISA and presence of cats

	Broiler			Laying hens		
	Reagents	Non reagents	Total	Reagents	Non reagents	Total
Presence of cats	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Yes	12 (14,5)	71 (85,5)	83 (100)	98 (35,8)	176 (64,2)	274 (100)
No	44 (11,7)	333 (88,3)	377 (100)	18 (23,7)	58 (76,3)	76 (100)
TOTAL	56 (12,2)	404 (87,8)	460 (100)	116 (33,1)	234 (66,9)	350 (100)

Broiler Chickens: $\chi^2 = 0,49$ (p=0,4821).
Laying hens: $\chi^2 = 3,92$ (p=0,0239); OR=1,8 (1,001 a 3,217)

(71%), which can be explained by the different technique associated with regional differences. Using the same technique, Dubey et al. (2006) determined the prevalence of *T. gondii* antibodies in chickens from 50 small farms in the Amazon region, finding 66% of seroreagent animals. These data show that the occurrence of anti-*T.gondii* seroreactive animals in free-ranged chickens increased in different regions of Brazil. These results were expected, since these animals were raised in direct contact with soil, while those raised in cages were not exposed to the sources of infection found in the environment. In an industrial scale egg-producing farm, some factors, such as proper hygiene measures, treated water, absence of cats and effective control of rodents, almost eliminate the contact of chickens with the parasite; thus making infection very unlikely. It is worthwhile noticing the high number of posture animals raised under confinement with positive reaction to *T.gondii* antibodies; indicating that management and hygiene were not ideal, therefore constantly exposing the animals to parasitism not only for *T. gondii*, but also by other agents.

The IFAT results for Group 1 indicated a low titer prevalence both in confinement and free-range chickens. As the raising system is extremely fast, we cannot consider this result a good marker for chronic or acute phase of T. *gondii* infection, because the age at which birds are slaughtered. Hence, it is not possible to infer whether there was enough time for titles to reach higher rates, indicative of acute infection, or if they were in the chronic phase of an infection acquired in infancy, which does not reduce the importance of these animals as a source of infection for other hosts. In Group 2 the increased frequency of low titers is noticeable. This fact associated with age (over 10 months) and confinement, suggests that chronic infection occurred in this population. For free-ranged posture chickens, since they are younger animals (aged from 3 to 5 months), the high frequency of high titers (1:1024 and 1:4096) could be indicative of acute infection in this population.

The oocysts from feces of domestic cats may represent a permanent source of *Toxoplasma* infection for birds (Literak & Hejlicek 1993, Dubey 2010). In broiler chickens, the contact with cats did not influence the distribution of reactive serum. As already discussed, some of the animals used in this study were from intensive chicken farming, which hinders the contact of poultry with the feces of definitive host. In Group 2 the presence of cats was observed in 7 of 9 farms visited. Statistical analysis using chi-square test showed a positive correlation between the presence of these animals on farms and the number of positive birds, and the odds ratio indicated a 1.8 times higher risk of infection when birds were living on farms where the domestic cat was present. Livestock farming can be prone to rodent infestation as it provides unlimited amounts of shelter, water and food to commensal rodents. They are constantly present in poultry farms throughout the country and may be a source of *T. gondii* infection not only for cats but also for chickens, which may be infected by ingesting bradyzoites from cysts, present in rodent muscles. In farms of Group 1, owners reported rodent infestation control using active methods, such as traps and chemicals, instead of using cats for this purpose. However, despite the control measures, the presence of rodents was reported in all farms. There was a higher chance for chickens that live on farms infested with rodents to be positive for *T.gondii* antibodies, which is related not only to a possible ingestion of tissue from infected rodents, but also and mainly to the fact that in most of the properties (77.8%) the control of these animals was made by passive methods, through the cats. This was also observed with other species of animals such as pigs (Millar et al. 2008).

Water is considered to be an important way of disseminating human toxoplasmosis, as *T. gondii* oocysts can persist for a long time in the environment (Tenter et al. 2000, Bahia-Oliveira et al. 2003). The water supply of farms in both groups did not receive any sort of treatment and therefore could be a source of *T. gondii* infection. In egg-producing farms, it was noticed that, mainly in free-ranged system, this non-treated water was used by farmers and their families, both for direct consumption and for washing household utensils, posing them in a risk of infection with *T. gondii*.

Considering that chickens may act as sentinel for environmental contamination (Dubey et al. 2006) and the high frequency of seroreagents animals in the studied area, it was possible that owners and staff had a twofold risk of exposure to the factors of infection. Besides, apart from those considered common sources of infection for both species, another worrying factor was that the habit of eating eggs and/or meat from chickens rose on those sites was reported in all farms. In the case of poultry farms, where the typical family raising system prevails, the poultry meat was the only source of animal protein consumed by the family and its employees.

In some farms there was still the habit of giving or selling to the neighborhood the 'old' birds that had reduced egg laying. The practice could expose local people to the infection. The risk of human infection was extended further by handling the carcasses on their own farm or slaughterhouse, where the butchers and veterinarians got in direct contact with infected tissues (Daguer et al. 2004; Millar et al. 2007).

CONCLUSIONS

The occurrence of anti-*Toxoplasma gondii* antibodies was high in both groups (broiler and posture chickens).

Free-ranged chickens raised for egg production proved to be the most exposed group to *T. gondii* infection. This can be related to the fact that these animals stayed on the farms for longer periods in direct contact with possibly contaminated soil through the presence of domestic cats.

These results suggest that control of cat population in chicken farms, performance of appropriate hygiene procedures and health education are important measures to reduce the infection rates in the region studied.

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